Montana 2009 Avian Influenza Surveillance Project Report

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The emergence and spread of the highly pathogenic avian influenza (AI) H5N1-Asian strain (HPAI H5N1) in Asia, the Middle East, Europe, and Africa has elevated concern about potential expansion of the disease to North America. Such an event could have negative affects on the poultry industry, humans, and wild bird populations (World Health Organization 2007). The role of wild migratory birds in the movement and transmission of HPAI H5N1 is poorly understood and strongly contested (Krauss *et al.* 2007, Peterson *et al.* 2007, van Gils *et al.* 2007). Circumstantial evidence suggests wild waterfowl may introduce AI viruses in the low pathogenic form to poultry flocks (World Health Organization 2007) and some species of waterfowl may asymptomatically carry HPAI H5N1 to new geographical areas during long distance migration (Chen *et al.* 2006, Lvov *et al.* 2006, Al-Azemi *et al.* 2008, but see Weber *et al.* 2007). Molting, migration stopovers, and wintering grounds allow birds to exist in high densities and provide opportunities for the transmission of low pathogenic avian influenza (LPAI) viruses between species, and wild and captive birds (Olsen *et al.* 2006, Chen and Holmes 2009), which then may recombine or mutate into a highly pathogenic form (Scholtissek *et al.* 1978, Ungchusak *et al.* 2005, Dugan *et al.* 2008).

The U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (WS) and the U.S. Fish and Wildlife Service (USFWS) initiated and funded a nationwide avian influenza surveillance project for the early detection of HPAI H5N1 in 2006, which was continued annually. The surveillance included all four flyways, all states, and tribal lands in the United States. Montana was considered a top priority state because the Pacific and Central Flyways divide the state and it borders Canada. Montana Fish, Wildlife and Parks (FWP), WS, and USFWS conducted sample collections for the 2009 Montana AI surveillance project. The Montana Department of Livestock Diagnostic Laboratory (MDoL), National Veterinary Services Laboratory (NVSL), and the U.S. Geological Survey National Wildlife Health Center (NWHC) tested samples. The Tribal Nations and the Department of Public Health and Human Services were also collaborators. The objectives of the project were to employ multiple sampling strategies to maximize the chance of detecting HPAI H5N1, including sampling live and hunter-harvested waterfowl, conducting state-wide systematic mortality/morbidity transects, and collecting samples from wild bird mortality/morbidity events.

Sample Design

The Montana AI surveillance sampling strategy was an adaptive step-down approach from the U.S. Interagency Strategic Plan (Interagency Asian HPAI Early Detection Working Group 2006) and the Pacific and Central Flyway plans (Pacific Flyway Council 2006, Central Flyway Council 2006). The above plans suggested that $\geq$200 samples would be required to detect one positive HPAI H5N1 sample in a defined bird population of >1000 individuals with a 95% confidence interval at a disease prevalence of $\leq$1.5%.

Swab Sampling Surveillance

The criteria outlined in the 2006 Montana Sampling Plan (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006) stated that FWP and WS would collaboratively collect swab samples from live and hunter-harvested birds from identified species of concern. Methods used in 2006 included collecting only a cloacal swab sample from each bird; in subsequent years of surveillance an additional oropharyngeal swab was collected and placed in the same vial with a cloacal swab to amplify the sample (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Laboratory testing of AI samples in 2006 included combining up to five individual cloacal samples in a sample pool to initially screen for all influenza A viruses. The protocol for the screening of samples in 2007 changed to testing each swab sample individually rather than pooling samples. Target sample numbers varied across years to adjust for the increased testing costs associated with initial screening (2006: n=2000, 2007: n=1500, 2008: n=1600). The 2009 sampling criteria goal was to obtain a total of 1400 cloacal-oropharyngeal statewide samples, 600 of which were to be collected by FWP and 800 by WS. Cloacal and oropharyngeal sampling strategies were: 1) coordinating with USFWS National Wildlife Refuge waterfowl trapping and banding operations, 2) sampling hunter-harvested waterfowl at National Wildlife Refuges and on state-owned lands, and 3) trapping wild and semi-domestic waterfowl on urban ponds across the state (Figure 1).

Mortality/morbidity Surveillance

Mortality/morbidity samples were collected statewide by FWP in collaboration with USFWS throughout each year of the project. Weekly prospective mortality/morbidity surveillance was added in 2007 as an AI detection method to systematically survey species of concern across Montana (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Mortality/morbidity surveillance began in summer during 2007 and 2008, while spring surveillance was added in 2009 to capture shorebird migration. Transects were conducted at $\geq$ 6 sites through freeze-up on bodies of water supporting species capable of demonstrating clinical symptoms due to HPAI infection (U.S. Department of the Interior Fish and Wildlife Service 2008).

METHODS

Cloacal and Oropharyngeal Sampling

Cloacal and oropharyngeal sample design assumptions included 1) the populations of birds to be sampled were homogeneous and accessible, 2) HPAI H5N1 was uniformly distributed across bird populations, and 3) representative sampling would be random and unbiased. Because these assumptions could not be met for wild migratory waterfowl, sample sizes were increased and sampling was extrapolated across large landscapes for multi-state and flyway sampling efforts in an attempt to account for biases (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). Cloacal and oropharyngeal sampling was spatially distributed across Montana and temporally distributed from August through December. According to the Implementation Plan for HPAI Surveillance in Wild Migratory Birds in the United States (2009), approximately 30% of swab samples should be collected from resident or non-migrating waterfowl and the remaining 70% should be collected from migratory species upon arrival in fall through freeze-up. Specific species identified as potential carriers of HPAI but not expected to exhibit clinical disease were targeted for surveillance. In keeping with the goals of Implementation Plan for HPAI Surveillance in Wild Migratory Birds in the United States (2009), species of primary concern for the 2009 live and hunter-harvested bird surveillance in Montana included those that tested positive for LPAI H5 or H7 in previous years of AI surveillance, which included dabbling ducks. Tundra swan, trumpeter swan, lesser snow goose, Ross's goose, greater white-fronted goose were also considered species of primary concern, as they have demonstrated the ability to asymptomatically shed HPAI H5N1, as well as succumb to the disease (Brown *et al.* 2008, Kalthoff *et al.* 2008, Hars *et al.* 2008). These primary species move between Asia and North America and

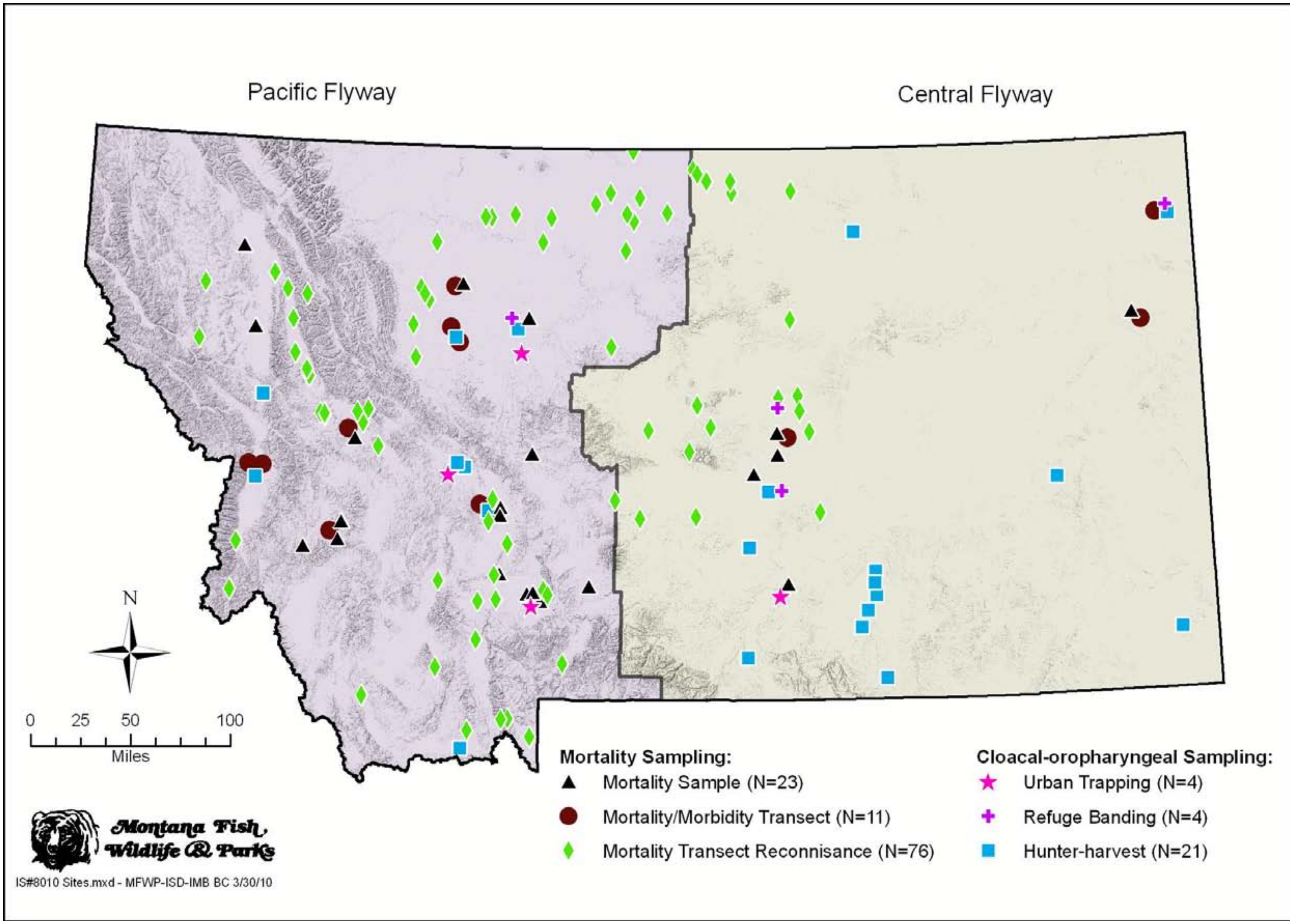

Figure 1. The Pacific and Central Flyways in Montana, and sampling sites for the 2009 Montana AI surveillance.

could contact Asian HPAI H5N1 directly (Alaska Interagency HPAI Bird Surveillance Working Group 2006). Diving ducks were considered secondary species from which samples should be collected. High numbers of most of these species migrate through the state and provide opportunity for sampling through refuge trapping and banding operations, waterfowl hunting, and urban trapping (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). Hybrid semi-domestic geese and mallards at urban ponds throughout the state served as sentinel species (Appendix 1).

Field Effort

Live bird AI sampling was conducted in conjunction with waterfowl banding at Benton Lake National Wildlife Refuge during September using methods approved by the U.S. Fish and Wildlife Service and Canadian Wildlife Service (1977). Net-launchers were used at three sites at Benton Lake and trapping efforts were rotated between sites. Waterfowl were banded by USFWS biologists and cloacal and oropharyngeal samples were taken by AI personnel. Sampled birds were then released. Swim-in traps also were employed at Medicine Lake, Lake Mason, and War Horse National Wildlife Refuges during September though no banding was performed at these refuges during 2009.

Urban wild and semi-domestic bird sampling began in the end of August and ran through November. AI personnel used swim-in traps at five urban ponds across the state to collect cloacal and oropharyngeal samples. Because swim-in traps required a flat surface covered by ≤1.5 feet of water, traps were set in water only at Bancroft Pond in Missoula and Gibson Pond in Great Falls. Swim-in traps modified for use on land were utilized at the Lewis and Clark Fairgrounds Pond in Helena, the MSU Pond in Bozeman, and the Overland Pond in Billings. Permission to trap was granted by city and/or county managers, while FWP Information and Education personnel worked with city managers to notify the public of trapping activities. The Bancroft Pond did not yield samples in 2009.

Hunter-harvested waterfowl sampling began in early October and ran concurrently with urban trapping through November. Waterfowl were sampled at Benton Lake, Bowdoin, Lake Mason, Lee Metcalf, Medicine Lake, Red Rocks Lakes, and War Horse National Wildlife Refuges, Freezeout and Canyon Ferry Lakes, Lake Helena, multiple sites on the Big Horn and Yellowstone Rivers, in the Flathead Valley, and on creeks and wetlands throughout the state. Hunter participation was voluntary and information about AI and the surveillance was distributed to hunters onsite. Sampling concluded when hunting diminished and as lakes froze.

Laboratory Testing

Cloacal-oropharyngeal samples were submitted to the MDoL and were tested using real-time reverse transcription-polymerase chain reaction (rRT-PCR). All samples were screened individually with a matrix gene primer/probe set designed to detect all influenza-A viruses. Samples testing positive were further analyzed to identify H5 and H7 subtypes (Spackman *et al.* 2002, Munster *et al.* 2009). Samples that screened positive or suspect for H5 or H7 were then sent to NVSL in Ames, Iowa, where confirmatory testing was performed for H5 and H7 subtypes using rRT-PCR and a standard rRT-PCR for N1. Virus isolation was also performed by NVSL on all samples to confirm AI virus isolates and determine whether or not H5 and N1 were linked in the same viral strain. All samples that produced positive results using virus isolation were then tested for pathogenicity using chicken inoculation studies and/or, if enough RNA was present in the clinical sample, a target amino acid sequence analysis was performed to determine virulence potential of the virus (U.S. Department of the Interior Fish and Wildlife Service 2006)

Sampling Effort

AI personnel collected 1400 cloacal-oropharyngeal samples toward the sampling objective for Montana during 2009; 651 samples were collected by FWP and 749 by WS (Table 1). One sample that tested LPAI positive, but H5 and H7 negative, was excluded from summarization and analysis because it was collected from a bird of unknown species and sex. Refuge trapping operations yielded 285 samples and urban trapping efforts produced 148 samples for a total of 433 live bird samples (31%). Hunter-harvested samples totaled 966. Sampling effort consisted of 85 total sampling days; refuge trapping produced 14 sample days, urban trapping yielded 8, while hunter-harvest produced 63. Sampling effort across all swab sampling methods resulted in overall means of 2.9 sample days/site and 16.5 samples/sample day across 29 sites. All methods produced similar numbers of samples/sampling day. As in previous years, Freezeout Lake was the most productive site, which yielded 22.3% (n=313) of the total swab samples collected.

Table 1. 2009 Montana AI surveillance swab sampling effort according to method.

	Sampling Method			
	Refuge trapping	Urban trapping	Hunter-harvest	Total
Number of samples	285	148	966	1399
Percentage of total samples	20	11	69	100
Total sample days	14	8	63	85
Number of sites	4	4	21	29
Sample days/sample sites	3.5	2.0	3.0	2.9
Samples/sample day	20.3	18.5	15.3	16.5

The Montana Sampling Plan (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006) called for high numbers of cloacal-oropharyngeal samples from primary species of concern and a focus on samples from secondary species to spread sampling effectively across species. Primary species comprised 84% (n=1158) of the total samples collected, of which 572 were mallards. Samples from mallards constituted 49% of the primary species and 41% of all cloacal-oropharyngeal samples. The remaining 51% of samples collected from primary species were collected across 12 species. Secondary species of concern comprised 17% (n=241) of the cloacal-oropharyngeal samples obtained (Table 6).

Montana cloacal and oropharyngeal sampling effort was spread temporally throughout fall in conjunction with refuge trapping operations 8/19 – 9/22, during the harvest of waterfowl 9/26 – 12/19, and urban wild bird sampling 8/27 – 11/24. Sampling peaked on 10/3, the opening day of waterfowl hunting in Montana, and ended in mid December as fall migration subsided. Primary species sampling began with mallards during urban trapping in late August and northern pintails during refuge trapping throughout September, while tundra swan, lesser snow goose, and Ross's goose sampling was conducted throughout the waterfowl hunting season. Additional primary and secondary duck species were sampled quite consistently throughout the hunting season. Sentinel birds (hybrid geese) were sampled at urban ponds in late September and November (Figure 2). Spatially, the highest proportion of cloacal-oropharyngeal samples was collected in the northeastern portion of the Montana Pacific Flyway at Freezeout Lake while the remainder of sampling was distributed relatively evenly across the rest of the state. Most samples from primary species of concern were collected Freezeout Lake and Benton Lake while secondary species sampling was distributed throughout Montana (Figure 3).

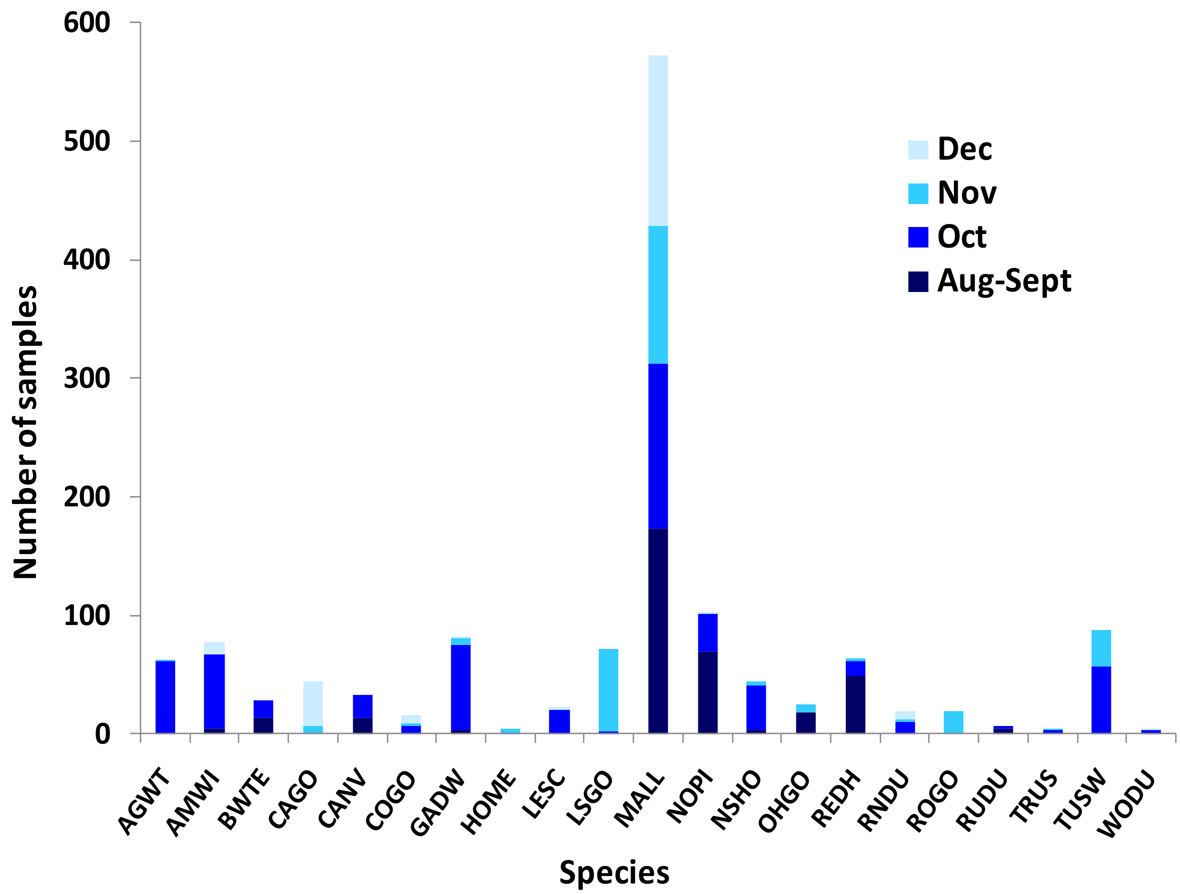

Figure 2. Temporal distribution of 2009 Montana AI cloacal and oropharyngeal sampling according to species. Species from which $\leq$2 samples were collected were excluded (American coot, bufflehead, common merganser: n=2 each; white-fronted goose: n=1).

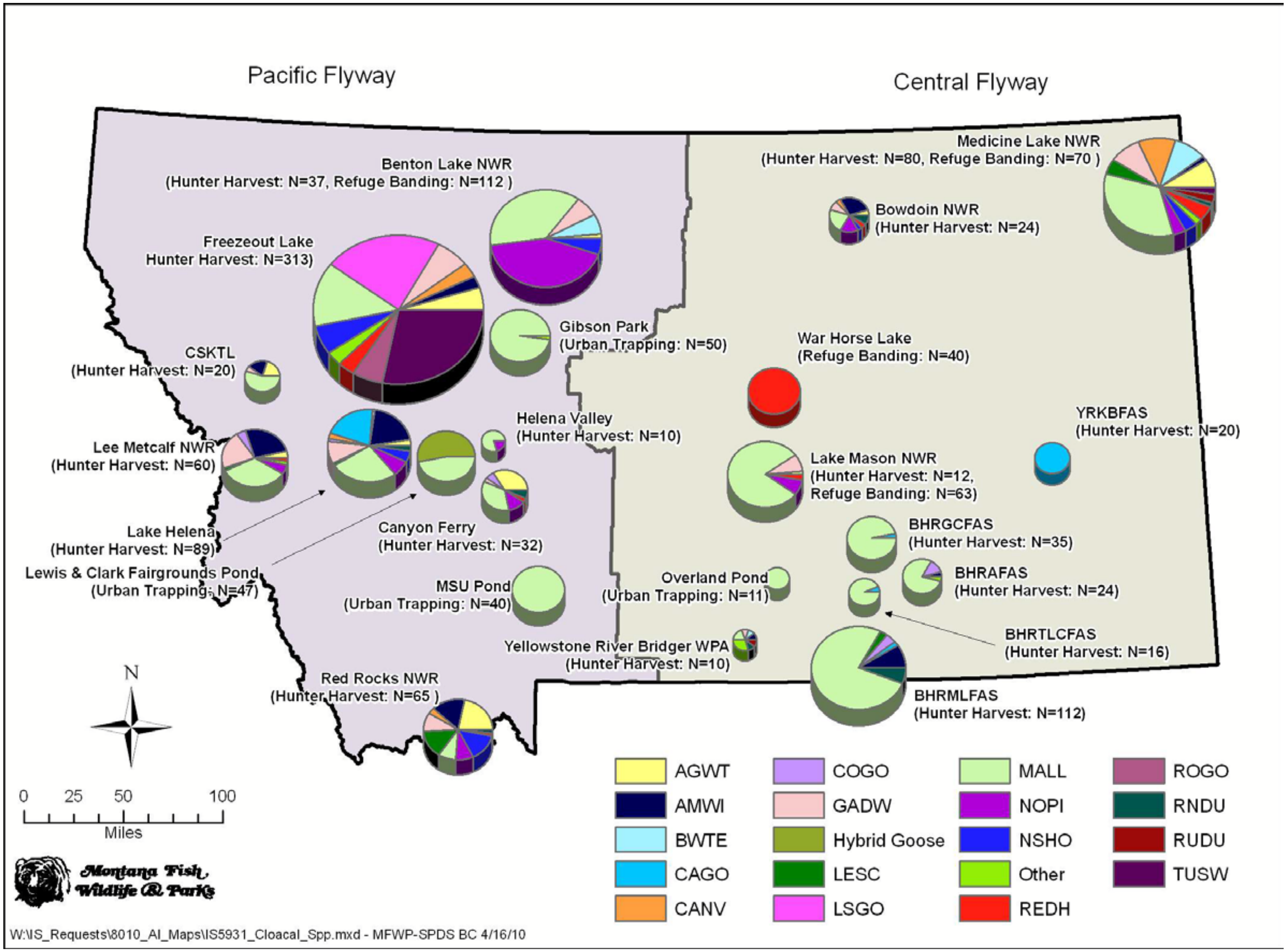

Figure 3. Spatial distribution of the 2009 Montana AI cloacal and oropharyngeal sampling according to species. The "Other" category combines all species from which ≤4 samples were collected (hooded merganser, trumpeter swan, wood duck: n=4 each; American coot, bufflehead, common merganser: n=2 each; white-fronted goose: n=1). Acronyms are used for the following sites: Big Horn River (BHR; General Custer: GC, Arapooish: A, Two Leggings Creek: TLC, Mallards Landing: ML, Fishing Access Site: FAS), Confederated Salish & Kootenai Tribal Lands (CSKTL), Yellowstone River Kinsey Bridge Fishing Access Site (YRKBFAS), National Wildlife Refuge (NWR), and Waterfowl Production Area (WPA). Species codes are located in Appendix 1.

Mortality/Morbidity Sampling

The 2009 Montana Sampling Plan Supplement specified the collection of $\leq$200 opportunistic mortality/morbidity samples during the 2009 sampling period. Reports made by the public were investigated according to the AI sampling criteria, which included consideration of the reported species as a potential concern for the presence of HPAI H5N1 and the circumstances under which the dead or sick birds were found. Morbid birds were euthanized in accordance with the Guidelines for Euthanasia of Non-domestic Animals (AAZV 2006). Bird carcasses suitable for disease testing found within 24 hours of death and euthanized birds were shipped for necropsy and disease testing at NWHC in Madison, WI.

Lab Testing

NWHC tested tracheal and cloacal swab samples and tissues by direct extraction. Testing procedures followed those described for cloacal-oropharyngeal sample testing and samples that tested positive for either H5 or H7 were sent to NVSL for confirmation (Spackman 2002, Munster et al. 2009).

Sampling Effort

A total of 42 FWP and USFWS mortality/morbidity samples were tested for AI by NWHC during the 2009 season. Carcasses from 22 species and 26 mortality events were collected statewide (Table 2). The 44 calls received by FWP about dead and dying birds yielded five mortality/morbidity sampling events and 11 events were discovered while performing mortality/morbidity transects. The remaining samples were fielded by agency personnel. Of the 37 birds categorized by age and sex, 21 were classified as hatch-year birds (6 females, 5 males, 10 undetermined) and 16 were classified as after-hatch-year birds (4 females, 9 males, 3 undetermined).

Mortality/Morbidity Transect Surveys

FWP AI personnel conducted weekly prospective transect surveys to systematically survey species of concern throughout the state of Montana for morbidity and mortality (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Species identified as sensitive to HPAI infection likely resulting in clinical disease and death were targeted for surveillance during spring and fall migration until freeze-up (U.S. Department of the Interior Fish and Wildlife Service 2008). Priority species included tundra and trumpeter swans, American wigeon, canvasback, lesser scaup, northern shoveler, redhead, ring-necked duck, and wood duck, as well as shorebirds, grebes, terns and gulls (Becker 1966, Brown *et al.* 2006, Brown *et al.* 2008). Reconnaissance was conducted throughout the Pacific and Central Flyways on lakes and wetlands in early and late fall to find sites for surveillance based on location, water conditions, access, and target species abundance. Once surveillance sites were established, surveys were conducted every 5-9 days and continually evaluated based on the presence of priority species. Surveys were performed consistently at six sites across the state and alternate locations were substituted when target species abundance declined due to migration (Figure 1). Surveillance was terminated at a site when total target species numbered $\leq$200, a site was inaccessible due to winter conditions, or the lake or wetland froze over.

Table 2. 2009 Montana AI mortality/morbidity samples tested for AI by NWHC according to species.

Species	Number of samples
American Coot	5
American Crow	1
American Robin	2
American White Pelican	2
Bald Eagle	3
Bohemian Waxwing	3
California Gull	1
Canada Goose	1
Double-crested Cormorant	1
Eared Grebe	1
Eurasian Collared-Dove	4
Gadwall	1
Great Blue Heron	1
Herring Gull	1
Lesser Scaup	1
Mallard	3
Ring-billed Gull	4
Rock Dove	3
Tundra Swan	1
Western Grebe	1
Western Tanager	1
White-winged Scoter	1
Total	42

Transects contoured within ten feet of the shoreline to detect morbidity and mortality events either by canoeing or walking. To record the presence of target species and index abundance, censuses were conducted with spotting scopes and high-powered binoculars from a single point on each transect that allowed maximum visibility to the observer. To avoid double counting during the performance of individual surveys, only numbers of each species counted upon initial sighting were recorded to yield a minimum number, and only counts of additional target species not seen during the initial census were added during the survey. Because it is likely bird populations were resampled across consecutive surveys, census data were reported as "bird observations". All symptomatic or dead birds of suitable quality were collected and tested for AI by submission of intact carcasses to NWHC following the protocols described above.

Sampling Effort

Performance of established mortality/morbidity transect surveys were conducted between 5/13 and 11/25, and reconnaissance on an additional 78 lakes and wetlands across the state began 5/18 and ended 11/18. A total of 234 weekly surveys were performed at 11 sites chosen according to the presence of target species, an increase of 110 surveys and a difference of three sites from the 2008 surveillance. Transect routes ranged from 2 to 10 km in length for a total of 51 km and averaged 5 (±2.73) km. Completed surveys ranged from 20 to 275 minutes and averaged 125 (±43.57) minutes for a total of 485 hours (Table 3). A total of 210,275 bird observations were recorded upon initial sighting of target species during the surveys, one third of which were ducks, geese, and swans. Approximately one fifth of the birds observed were gulls and terns, and the remaining tenth was comprised of grebes, shorebirds, and cranes (Table 4). Dead and sick birds found on transects totaled 151 and 8, respectively. The 146 carcasses identifiable to species were comprised of one gadwall, lesser scaup, and common loon, two herring gulls and Canada geese, six ring-billed gulls, 11 American white pelicans, and 85 American coots, 84 of which were found at Georgetown Lake. Thirteen of the carcasses collected on transects were sent to NWHC to test for AI and determine cause of death.

Table 3. 2009 Montana AI mortality/morbidity transect survey start and end dates, length and average survey times for complete surveys.

Transect	Date		Transect	Average (total)	Number
	start	end	length (km)	survey time (min)	of surveys
Brown's Lake	6/4	9/13	9	145	14
Canyon Ferry, Pond 2	5/15	11/25	6	180	29
Eyraud Lakes	5/14	11/23	5	95	30
Freezeout Lake, Pond 6	5/13	9/25	3	85	22
Freezeout Lake, NW Bay	10/3	11/23	4	80	8
Fox Lake	5/14	8/24	2	105	14
Georgetown Lake	5/26	11/23	4	130	27
Lee Metcalf, Otter Pond	9/4	11/24	2	90	13
Medicine Lake, Sayer Bay	5/22	11/16	4	140	24
Pablo Reservoir	6/3	11/23	10	150	25
Yellow Water Reservoir	5/15	11/23	2	115	28
Total	5/13	11/25	51	125 (29,110)	234
Transect reconnaissance	5/18	11/18	---	55 (5,050)	96

Table 4. Montana 2009 mortality/morbidity transect survey bird observations according to family.

Family	Number counted	(%)
Anatidae (ducks, geese, swans)	140,784	(67)
Laridae (gulls, terns)	46,806	(22)
Podicipedidae (grebes)	16,002	(8)
Scolopacidae (sandpipers, phalaropes)*	4,153	(2)
Charadriidae (plovers, killdeer)	1,443	(1)
Recurvirostridae (avocets, stilts)	981	(>1)
Gruidae (cranes)	63	(>1)
Pelecanidae (pelicans)	42	(>1)
Gaviidae (loons)	1	(>1)
Total	210,275	(100)

*Includes curlews, dowitchers, godwits, sanderlings, snipes, willets, yellowlegs, unidentified shorebirds.

Data Management, Reporting of Results, Statistics

AI personnel entered cloacal and oropharyngeal sampling data directly into the NVSL national web-based database system. NVSL reported all cloacal-oropharyngeal sample results through the same database, which included H5, H7, and N1 screening results, as well as LPAI subtype and pathogenicity. All 2009 cloacal and oropharyngeal data and results were then uploaded to FWP's existing AI database. NWHC reported mortality/morbidity results directly to FWP, which contained the outcome of AI and additional disease testing, and cause of death when possible. AI mortality/morbidity transect survey and carcass data and results were entered into FWP databases. Confidence intervals were calculated for the proportion of LPAI positive cloacal-oropharyngeal swab samples according to species (R Core Development Team, 2006). Using the Agresti-Coull interval, the assumptions were 1) sampling was random or at least representative of the entire population, 2) LPAI rates were the same temporally, spatially and across trapping methods, and 3) there was no measurement error. Confidence intervals for LPAI positive cloacal-oropharyngeal swab samples by sex and age classes for individual species were not calculated due to the large differences in the proportion of LPAI positive samples within each sex and age class.

RESULTS

While AI virus was found in samples, HPAI H5N1 was not detected in Montana during the 2009 surveillance. Because the AI surveillance did not focus on the detection of LPAI, samples that tested LPAI positive but H5 and H7 negative were not tested with virus isolation to determine AI subtype.

Cloacal-oropharyngeal Samples

LPAI Results

Of the total 1399 cloacal-oropharyngeal samples included in the 2009 analysis, 248 (18%) samples tested positive for LPAI. Though the hunter-harvest method produced 69% of the samples for AI testing and 52% of the overall LPAI positive results, refuge trapping yielded the highest percentage of LPAI positive samples within sample collection method (40%: Table 5).

Table 5. 2009 Montana LPAI positive cloacal-oropharyngeal sample numbers and percentage according to method of sample collection.

Sampling method	Number of samples	Number of LPAI positive samples	Percentage LPAI positive samples of method total
Hunter-harvest	966	128	13%
Refuge trapping	285	113	40%
Urban trapping	148	7	5%
Total	1399	248	18%

According to temporal analysis by sex and age class, the proportion of hatch-year females that tested LPAI positive during September and October was highest among all sex and age classes. Peak for all sex and age class among LPAI positive samples was in September (Figure 4).

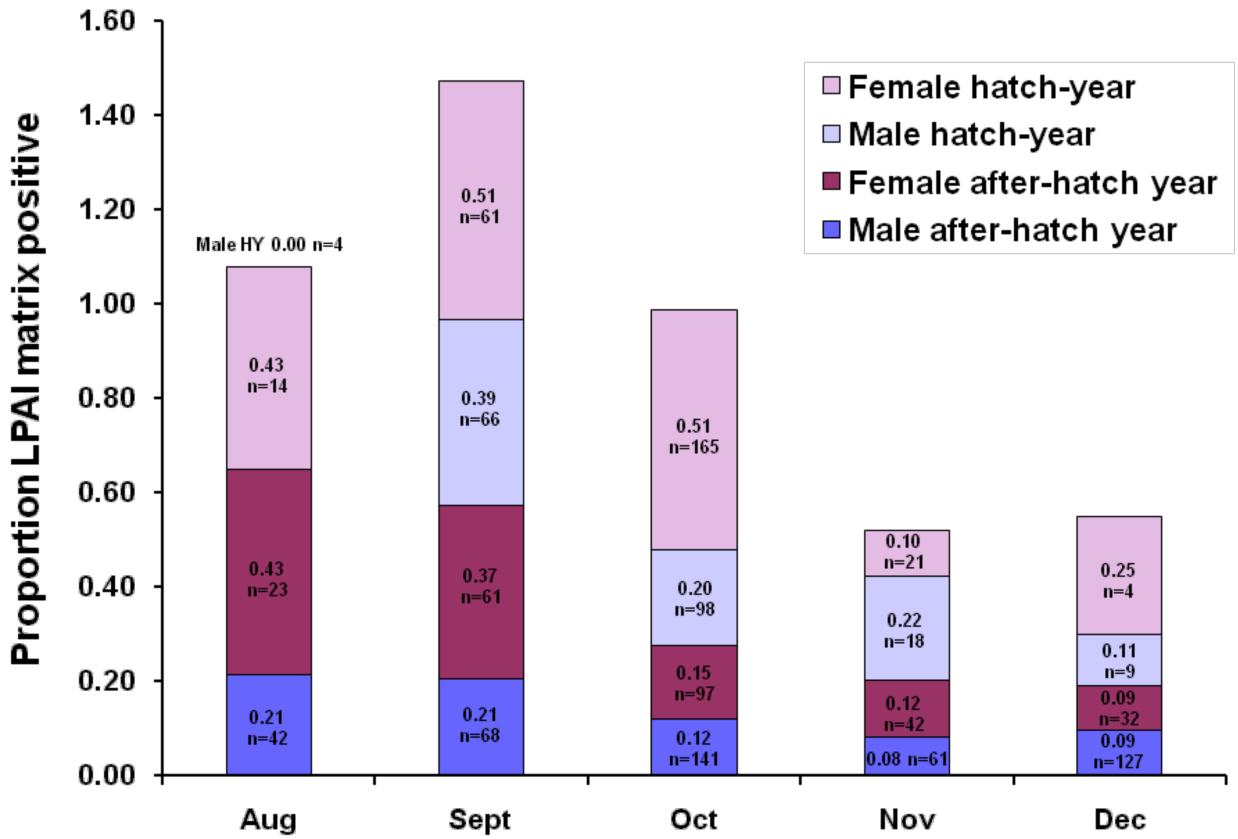

Figure 4. Proportion of 2009 LPAI positive cloacal-oropharyngeal samples according to known sex and age classes (n=1145).

Known sex and age classes across all sampled species and methods were pooled for species-specific analysis. The highest proportion of LPAI positive samples within the primary species of concern was northern pintail (0.44). Among other primary species, the proportion of LPAI positive samples for blue-winged teal, American green-winged teal, northern shoveler, and mallard was .038, 0.35, 0.25, and 0.20, respectively. Samples from the remaining seven primary species analyzed resulted in a proportion of LPAI positive samples below the 0.15 average among all species. Samples from secondary species of concern that resulted in a proportion of LPAI positive samples above the average among all species were hooded merganser (0.50, however, note the small sample size of n=4) and common goldeneye (0.19), while the rest were below the average among all species (Table 6). Four species were excluded from analysis due to small sample sizes (American coot, common merganser, bufflehead: n=2; white-fronted goose: n=1).

Table 6. Proportion of 2009 Montana cloacal-oropharyngeal swab LPAI positive samples according to species using the Agresti-Coull interval. Mean= proportion of LPAI positive samples within species, Lower CI= lower 95% Confidence Interval, Upper CI= upper 95% Confidence Interval, X= number of LPAI positive samples within species, N= number of birds sampled within species.

	Species (n=21)	**Mean**	**Lower CI**	**Upper CI**	**X**	**N**
Primary species	Northern Pintail	0.44	0.35	0.53	45	103
	Blue-winged Teal	0.38	0.23	0.56	11	29
	American Green-winged Teal	0.35	0.24	0.47	22	63
	Northern Shoveler	0.25	0.14	0.40	11	44
	Mallard	0.20	0.17	0.24	116	572
	American Wigeon	0.08	0.03	0.16	6	77
	Lesser Snow Goose	0.08	0.04	0.17	6	72
	Tundra Swan	0.08	0.04	0.16	7	88
	Gadwall	0.05	0.02	0.12	4	82
	Ross's Goose	0.05	0.00	0.26	1	19
	Trumpeter Swan	0.00	0.00	0.55	0	4
	Wood Duck	0.00	0.00	0.55	0	4
Secondary species	Hooded Merganser	0.50	0.15	0.85	2	4
	Common Goldeneye	0.19	0.06	0.44	3	16
	Ruddy Duck	0.14	0.01	0.53	1	7
	Lesser Scaup	0.13	0.04	0.33	3	23
	Redhead	0.09	0.04	0.19	6	64
	Ring-necked Duck	0.05	0.00	0.26	1	19
	Canada Goose	0.05	0.00	0.16	2	44
	Canvasback	0.03	0.00	0.17	1	33
	Hybrid Goose	0.00	0.00	0.34	0	25
	Total	0.15	-----	-----	248	1392

H5, H7, and N1 Results

Twenty-eight of the cloacal-oropharyngeal samples analyzed in 2009 tested H5 positive, all of which produced N1 negative results. One sample tested positive for H7 and was typed as LPAI H7N3.

Mortality/Morbidity Samples

Of the 42 mortality/morbidity samples submitted for examination to NWHC, three American coots produced presumptive LPAI positive results and negative results for H5, H7, and N1. Cause of death for mortality events were reported to individual submitters by FWP and were not included in this report.

DISCUSSION

AI virus in low pathogenic form was detected in Montana samples as expected, while HPAI H5N1 has not been found to date in Montana or elsewhere in North America. Twenty-eight birds sampled with cloacal-oropharyngeal swabs tested H5 positive and N1 negative.

Within sampling methods, hunter-harvest swab sampling produced the most samples (69%) and less than the average 15% LPAI prevalence across all methods (13%), while refuge trapping yielded the highest percentage of LPAI positive samples (40%). The highest proportion of LPAI positive samples occurred in September and then declined throughout fall, consistent among all years of AI surveillance in Montana. Timing of refuge trapping verses hunter-harvest and urban trapping sampling may partially explain this difference. Several studies have shown that AI is more prevalent in early fall and decreases as fall migration proceeds (Stallknecht 2003, Gilbert *et al.* 2006). Changes in LPAI concentration may be due to a combination of premigration density of waterfowl with the high recruitment rate of immunologically naïve juveniles in early fall, while subsequent declines in LPAI may be a result of increased flock immunity and progressive dispersal of bird populations (Stallknecht 2003, Gilbert *et al.* 2006). The use of different trapping methods may also contribute to the differing low pathogenic AI results.

Northern pintails tested in Montana for AI during the 2009 surveillance produced the highest prevalence of LPAI positive results (44%) within the primary species of concern. Recent studies have shown that northern pintails carry numerous strains of LPAI at some of the highest prevalences among water bird species (Hinshaw *et al.* 1980, Runstadler *et al.* 2007, Ip *et al.* 2008, Parmley *et al.* 2008). Hatch-year northern pintails tested in Alaska produced higher prevalences than the adults, while hatch-year males and females differed little (Ip *et al.* 2008). In Montana, the 2009 female northern pintail LPAI prevalence was 49%, higher than the male prevalence of 37%. Age classes also differed; hatch-year northern pintails produced higher LPAI prevalences than adults, 50% and 31%, respectively. The highest prevalence among the sex and age classes was found in hatch-year females (53%, n=38).

Success of wild live and hunter-harvested bird sampling, as well as mortality/morbidity sampling, depends on the availability of the species and numbers of birds during migration. The timing of migration can be affected by many factors, including climate and weather patterns (Blokpoel and Richardson 1978, Nichols *et al.* 1983, Harmata *et al.* 2000), age of the migrants (Hepp and Hines 1991), population size (Nichols *et al.* 1983), and bird body mass, especially in hatch-year birds (Owen and Black 1989). It was important to obtain high numbers of hatch-year bird samples because that age class likely contained the highest prevalence of AI viruses during their first fall migration (Olsen *et al.* 2006); this was accomplished during each year of Montana AI surveillance. While mallard was the most abundant and available species in Montana and was sampled across the sampling season, an effort was made to limit mallard sampling to maximize sampling of other target species. Urban trapping provided the greatest temporal flexibility among swab sampling methods, as sampling could be conducted according to schedule rather than opportunistically, however, it also afforded the least diversity of species (n=3). Conversely, hunter-harvest sampling was difficult to allocate temporally while it provided the most species diversity (n=25); 15% of the total hunter-harvest samples were collected during the first weekend of the waterfowl hunting. Refuge trapping provided eight species and was concentrated during the month of September at four National Wildlife Refuges.

To distribute sample collection temporally during the 2009 surveillance, emphasis was placed on sampling wild sentinel birds at urban ponds throughout the sampling period, northern pintails during refuge trapping in early fall, and tundra swans and lesser snow geese during hunter-harvest later in fall.

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APPENDIX I.

Target Species and Species Codes for Cloacal-oropharyngeal Sampling

	Species (n=25)	Species Code
Primary species	American Green-winged Teal	AGWT
	American Wigeon	AMWI
	Blue-winged Teal	BWTE
	Gadwall	GADW
	Greater White-fronted Goose	GWFG
	Lesser Snow Goose	LSGO
	Mallard	MALL
	Northern Pintail	NOPI
	Northern Shoveler	NSHO
	Ross’s Goose	ROGO
	Trumpeter Swan	TRSW
	Tundra Swan	TUSW
	Wood Duck	WODU
Secondary species	American Coot	AMCO
	Bufflehead	BUFF
	Canada Goose	CAGO
	Canvasback	CANV
	Common Goldeneye	COGO
	Common Merganser	COME
	Hooded Merganser	HOME
	Hybrid Goose	OHGO
	Lesser Scaup	LESC
	Redhead	REDH
	Ring-necked Duck	RNDU
	Ruddy Duck	RUDU